

The University of Chicago

WATER RELATIONS IN BRYOPHYLLUM
CALYCINUM SUBJECTED TO
SEVERE DRYING

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
1937

By
WALTER BURCHARD WELCH

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WATER RELATIONS IN *BRYOPHYLLUM CALYCINUM* SUBJECTED TO SEVERE DRYING

WALTER BURCHARD WELCH

(WITH TWO FIGURES)

Introduction

Bryophyllum calycinum Salisb. will reproduce vegetatively by means of plantlets formed in the notches of the vegetative leaves. This reproduction, called regeneration by LOEB (9), has been rather thoroughly treated by LOEB (10), REED (17), BRAUN (1), CHILD and BELLAMY (2), and others. A great deal of attention has been paid by these authors to the reproduction of the young plants, but little attention has been given the water relations in the parent leaf during their formation and growth.

In most of the work reported the leaves, when removed from the plant, were placed in contact with water or at least in a moist atmosphere. Under laboratory conditions, without extraneous water, the plantlets appear on the margin of all portions of the leaf without regard to polarity (fig. 1, A, B). The leaves, when hung up by their petioles, produce young plants on the basal half as well as on the apical half, but when placed with one edge in water or in moist soil the shoots will appear only where the leaf is in contact with the water or moist soil.

The parent leaf may dry until it becomes brittle at its central portion, yet the young plants along the margin will still be turgid and continue to increase in size. Since this leaf is capable of living for a long period and reproducing new tissues without additional water being supplied to it, it was proposed that a study be made of the water relations of the parent leaves and their plantlets. A great number of phenomena might be studied in connection with the ability of these leaves to withstand severe drying: osmotic concentration of the juices of the leaves and plantlets, electrical conductivity of these juices, hydration of the ions present in the solutions, relationship of the freezable and unfreezable water to the total water, the thickness of the cuticle, the distribution of stomata, and so on. The work presented in this paper will deal with the freezable and unfreezable water in their relation to the total water, the thickness of the cuticle, and the distribution of stomata.

Unfreezable water has attracted a great deal of attention among plant physiologists who were interested in cold resistance or the ability of plants to become hardened against killing at low temperatures. ROSA (19) found that in cabbage the rate of decrease in the percentage of freezable water during hardening coincided with the rate of hardening of plants against killing by low temperatures. GREATHOUSE and STUART (6) found that

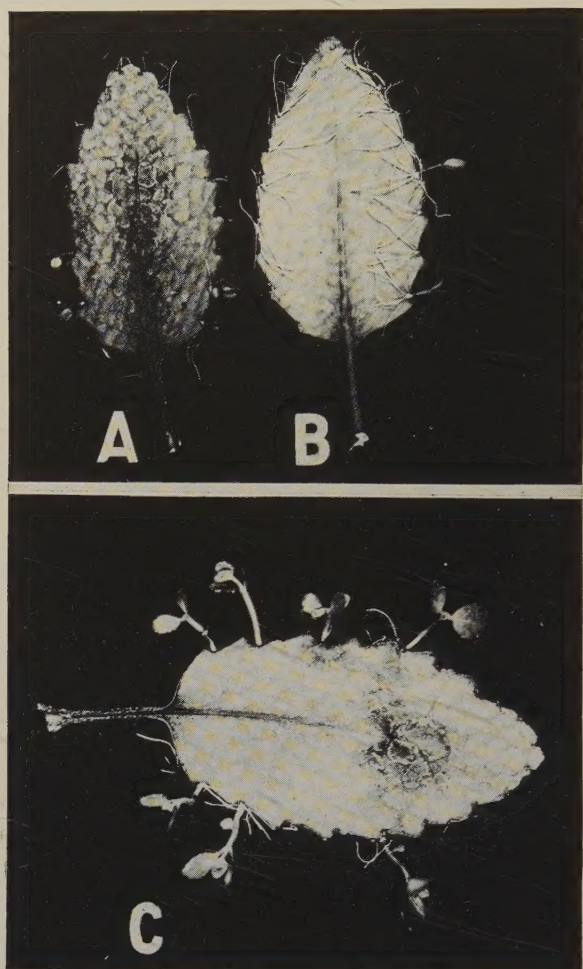


FIG. 1. A. Upper surface of leaf of *Bryophyllum calycinum* after 4 weeks of drying, showing plantlets in notches of leaf ($\frac{1}{2}$ natural size). B. Lower surface of leaf showing maximum production of roots at 4 weeks ($\frac{1}{2}$ natural size). C. Upper surface of leaf in A showing plantlets and drying central region after 6 weeks ($\frac{3}{8}$ natural size).

Ohio and French varieties of red clover could be differentiated as to cold hardiness by the percentage of unfreezable water in the fresh tissues. STARK (21) demonstrated that in general the capacity of an apple twig to retain water against freezing is associated with winter hardiness. He could not, however, grade the hardy and tender varieties in any ascending or descending order on the basis of their ability to retain water in the unfrozen state. In *Pinus rigida* there was only about 5 per cent. more water in the

leaves in the summer than in the winter, and MEYER (13) states that there is no evidence that "bound" water plays any rôle in cold resistance in this species. He suggested "that the basis of cold resistance lies in some as yet not understood, physio-chemical properties of the protoplasm, which probably cannot be discovered by the gross measurements which are generally employed at present." DUNN (3) found that *Bryophyllum*, subjected to a temperature of -1.1°C ., would temporarily build up a resistance to this cold temperature to such an extent that a certain number of plants would not be killed after exposure for 15 hours. Surviving plants were propagated for six generations by leaf cuttings. There was a rise in the number of survivors after three generations of cuttings but after six generations there was a return to the original number.

From these and other reports it seems possible that there is a positive correlation between unfreezable water and cold resistance in some species or varieties; but in other species or varieties such a positive correlation between unfreezable water and cold resistance may not be demonstrable.

The terms "free" and "bound" water, used by the earlier investigators, no longer convey the idea investigators wish to express. It is quite possible that under a given set of conditions and at a given time, all or nearly all of the water in a plant may be "bound," and under other conditions and at another time nearly all of the water may be "free." It would be better to refer to the "bound" as unfreezable water and the "free" as freezable water. It then becomes necessary to define the conditions under which the freezing, in the heat of fusion method of determination of freezable water, is carried out. The water is held in the tissues, cells, or parts of cells against freezing in equilibrium with the surrounding atmosphere, or any other tissues, cells, or parts of cells with which they may come in contact. This has been pointed out by GREATHOUSE (5) in his work on red clover roots and tissues of the potato tuber. He found that there was a greater amount of water frozen in the tissues at -50°C . than at -22°C . JONES and GORTNER (7) found that as much water was frozen out of gelatin at -6°C . as was frozen out at -50°C . They suggested that plant tissues should behave as did the gelatin, but the experiences of GREATHOUSE show that there is a freezable-unfreezable water equilibrium that has a different value for every temperature used.

In this work on *Bryophyllum* the temperature of -20°C . was chosen as the one at which the freezable-unfreezable water equilibrium should be measured. There is nothing to suggest that -50°C . would be the lower limit at which the freezable water can be measured; any obtainable temperature above absolute zero might be chosen for such measurements. Since an equilibrium relation is being dealt with, the most important feature is the maintenance of the selected freezing temperature for sufficient time to

permit an equilibrium to become established, and to hold that temperature constant within a range of $\pm 0.25^{\circ}\text{C}$.

The problem of drought resistance or of resistance to very severe drying in relation to the unfreezable water content of the tissues has received much less attention than the relation of unfreezable water to cold resistance. VASSILIEV and VASSILIEV (23) found that in general the most conspicuous feature in which a wheat plant, that had been hardened to resist drought, differs from a normal one is in the greater accumulation of hemicelluloses and sugars, chiefly sucrose. The plants which had been exposed to drought by withholding water for 18 days, but were later given an ample water supply, were found to be deficient in water after 8 days of irrigation. They were, however, higher in most forms of carbohydrates except the monosaccharides. This water-deficient, high-carbohydrate condition became more or less "fixed" in plants that had recovered from the drought conditions.

The concentration, osmotic pressure, and "bound" water of the juices of some of the cultivated and some of the wild species of grasses, were found by NEWTON and MARTIN (16) to increase with the progress of maturity. The unfreezable water was found to increase markedly more rapidly than the osmotic pressure and appeared to be more stable. LEBEDINCEV (8) used unfreezable water measurements to determine the water-retaining capacity of xerophytes and mesophytes growing under xerophytic conditions in a semidesert region. She has shown that xerophytes are distinguished by having a higher water-retaining capacity than mesophytes under the same conditions. If the plants were subjected to repeated wiltings there was an increase in the water-retaining capacity which was maintained by the plants after recovery from wilting.

Bryophyllum is usually not considered a xerophyte unless MAXIMOV'S (11) criterion, "the main peculiarity of xerophytes is the capacity of enduring permanent wilting without harm or without harm to their subsequent development," is used. The "wilting without harm to their subsequent development" is true only of leaves that are removed from the plant while they are in a turgid condition. If the leaves become dry on the plant and then fall or are removed, they will not produce plantlets on the margin even though roots may have been present at the notches of the leaves while they were still on the plant.

The series of determinations reported in this paper were undertaken to throw light, if possible, on the significance of the unfreezable water in both the parent leaf and the regenerating tissues, and the significance of any shift or redistribution of total water between parent leaf and its plantlets, with special reference to severe drying of the parent leaf and the ability of its regenerating offspring to persist and develop after the parent leaf had partially dried.

Materials and methods

The *Bryophyllum calycinum* used was that which MEHRLICH (12) has called the Chicago variety. This variety has been grown in the University of Chicago greenhouses for a number of years. Although it has been observed to flower no seeds have been obtained. The plants were vigorously grown under greenhouse conditions until they had produced eight sets of leaves, then the leaves from the fourth and fifth nodes were removed. In preliminary tests, these leaves from the fourth and fifth nodes were found to produce more shoots per leaf than the leaves from any other node. The leaves were allowed to dry in screen-wire trays on a laboratory table for as much as 18 weeks. After a week, the leaf became limp; after 2 weeks, roots appeared at the notches; and after 4 weeks the shoots of the plantlets appeared (fig. 1). The development of the roots and shoots has been adequately described by NAYLOR (15) and YARBROUGH (24). The leaves, after removal from the plant, did not come in contact with any extraneous water except that of the laboratory atmosphere.

The leaf continued to dry until at 10 weeks the central portion, indicated by the shaded area in figure 2, had become tough and brown while the tissues along the edge and base became even more turgid and remained green. The solid line in figure 2 indicates the manner in which the leaf was cut to separate that portion enclosed by the line, hereafter called the central region, from that portion outside, hereafter called the edge and base. This latter portion includes the petiole in all cases.

The method used to determine the unfreezable water was that of RUBNER (20) as modified by ROBINSON (18). Most of the equipment was that described by ROBINSON. The cold chamber was well insulated and deep enough that a minimum change in temperature at the level of the material was observed when the lid was removed. The temperature was maintained at $-20^{\circ}\text{C.} \pm 0.25$. Two thermopiles were made of no. 30 gauge double-silk insulated copper and constantan wire, on which two coats of shellac had been brushed. The thermopile used in the cold chamber to check the temperature was one of only five junctions, while the one used in the calorimeter had twenty junctions and produced a potential of 763 microvolts per degree difference in temperature. The "cold" junction in each case was in a thermos bottle filled with a mixture of crushed ice and ice water. The potential in microvolts was read from a Leeds and Northrup type K potentiometer.

The calorimeter was a highly evacuated, highly silvered Dewar flask, 12 mm. in diameter and 65 mm. deep. The flask was sealed into a three-fourths-inch layer of cork with sealing wax at either end, and finally wrapped in tape and varnished. This insulation made it practically impossible to touch the edge of the calorimeter with the fingers during the transfer of the

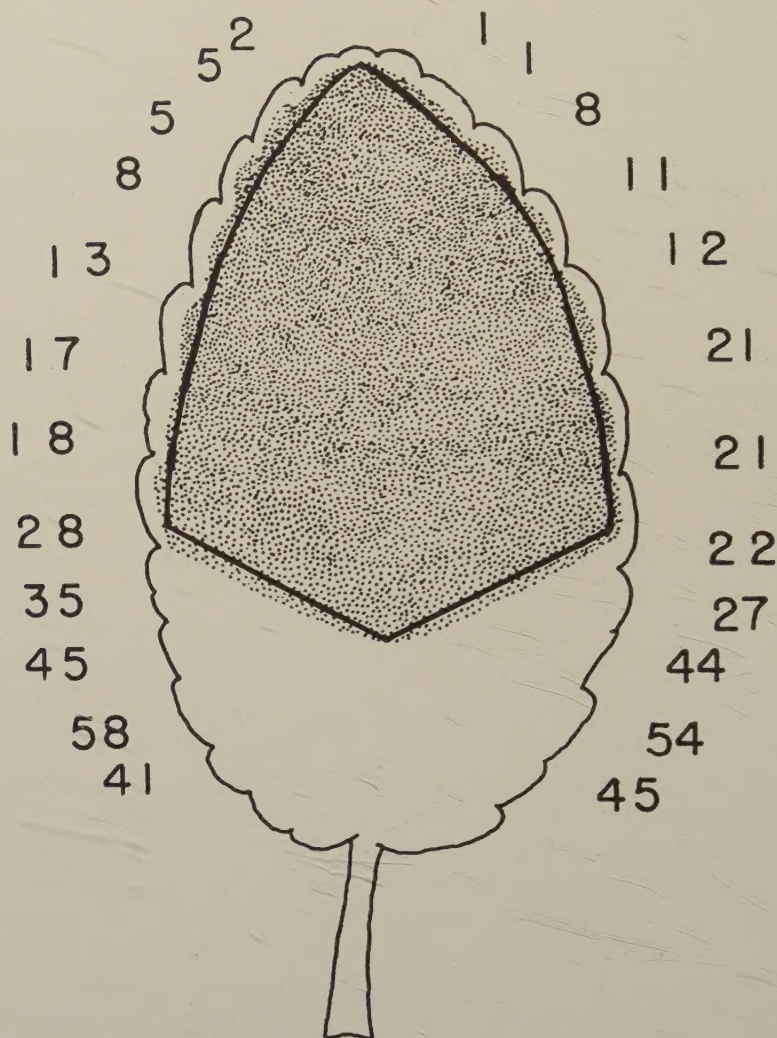


FIG. 2. Shaded portion of leaf of *Bryophyllum calycinum*, which is tough and brown, showing extent of drying at 10 weeks. Solid line shows how leaf was cut to separate central portion from the edge and base. Numbers at margin represent the plantlets counted on 100 leaves varying in age from 8 to 12 weeks.

material from the cold chamber to the calorimeter. The calorimeter was placed in a water bath maintained at 30° C.

A spiral glass stirrer was used to mix the water in the calorimeter. To the stirrer motor a flexible cable was attached which was held in place by two glass bearings in ring stand clamps. The lower end of the cable was attached to a steel bearing which could be fastened rigidly in place. The

glass stirrer fitted into a chuck on the steel bearing shaft. The motor and bearings were so mounted on a hollow rod that the stirrer could be swung in and out of the calorimeter in minimum time; and when not in the calorimeter, the stirrer dipped into a flask of distilled water in the water bath. Owing to the heating of the motor and the bearings, factors from 1.196 to 1.226 had to be used for the thermal capacity of the calorimeter in a continuous series of ten determinations. Never more than ten determinations were made in any one series.

The freezable water was calculated by use of the formula presented by ROBINSON (18) :

$$X = \frac{FN(T_2 - T_3) - (WSR + W_1S_1R)}{80 - \frac{T_1}{2}}$$

in which F = correction factor for the thermal capacity of the calorimeter

N = volume of water in cc. used in calorimeter (10 cc.)

T₂ = initial temperature of water in calorimeter

T₃ = final temperature of water in calorimeter

W = total weight of the material

S = specific heat of the material

R = range of temperature between T₁ and T₃

W₁ = weight of tin-foil cup

S₁ = specific heat of tin foil which is 0.05

T₁ = initial temperature of material in cold chamber

This being below zero appears to have a minus sign but the formula is so constructed that the sign may be disregarded.

X = freezable water

The unfreezable water is found by subtracting the freezable water from the total water present. The specific heat of each specimen was calculated from the specific heat of the dry material, which was found to be 0.0871, and the specific heat of water, 1.

The plant material was chopped with a razor blade and ground to a paste in a mortar. This thoroughly ground material was then placed in a tin-foil cup in a numbered vial and stoppered. The samples ranged in weight from 0.25 to 0.85 gm., but the usual sample weighed about 0.5 gm. After freezing, the cups were removed from the vial and the tops bent down in such a manner as to form waterproof balls, after which they were replaced in the cold chamber and frozen for at least one hour.

The vial was removed from the cold chamber by grasping the stopper with the fingers of the right hand. The upper end of the vial was held with the fingers of the left hand only long enough to remove the stopper and slide the specimen into the calorimeter. After thawing, the material was dried in a vacuum oven at 47° C., using a pressure of 0.04 atm.

Results

DETERMINATIONS OF FREEZABLE AND UNFREEZABLE WATER

The data were obtained from three series of experiments. The first series was performed to determine the distribution of the total, freezable, and unfreezable water in the whole plant. This information was necessary not only to establish the normal relationship in the leaves and stem, but also to determine how the leaves at the fourth and fifth nodes compared with the other leaves.

The second series was designed to determine the relationship of total, freezable, and unfreezable water in the whole leaf, with its attached plantlets, during the period of drying. The data from this series were to serve as a guide for the third series of measurements.

In the third series the purpose was to determine the relationship of the water, as in the second series, in the different parts of the leaf and the attached plantlets. In this latter series of measurements it was thought that some light might be thrown on the relation between the freezable and unfreezable water in the parent leaf and in the young plants with special reference to any shift or redistribution of water there may be between the two during the severe drying.

SERIES 1. DISTRIBUTION OF TOTAL, FREEZABLE, AND UNFREEZABLE WATER IN THE WHOLE PLANT

The plants used in this series of experiments were vigorously growing individuals that had nine sets of leaves below the apical bud, and a portion of the stem below the ninth set of leaves that was bare. It was impossible to separate the apical bud from the first internode and the first set of leaves and obtain a specimen that would be comparable in weight to the other samples of the stem internodes and the leaves. Therefore the apical bud, first internode, and the first set of leaves were chopped, ground, and frozen as a single sample. The second set of leaves was then removed and treated in the same manner; then the second internode, and so on down the stem.

The lowermost portion of the stem was made up of six very short internodes without leaves, and was treated as a single sample. The determinations on this portion of the stem were entered in table I as the basal internodes. This lower portion of the stem was very woody and rather difficult to reduce to the same degree of fineness as the other samples.

As may be seen from the data of column 2, table I, the first six internodes contained more total water and more freezable water than was found in the leaves attached at the first six nodes, when calculated on the dry weight basis. Internode three contained the largest amount of water of any part examined, and the basal internodes the least. From internode three to internode eight

there was a gradual decrease in total water from 10.385 gm. of water per gram of dry weight to 2.692 gm., and on the same basis the freezable water decreased from 9.706 to 2.368 gm. of water (column 3, table I).

The second and third internodes (column 5, table I), contained a smaller percentage of unfreezable water, 5.62 and 6.53 per cent., respectively, than the leaves at the second and third nodes, 10.40 and 8.66 per cent. The internode contained 7.65 per cent. of unfreezable water while the leaves at the fourth node had only 5.69 per cent. This relation, the higher percentage of unfreezable water in the stem than in the leaves attached to that portion of the stem, was observed in all parts examined, below the fourth node. That portion of the stem referred to as the basal internodes had only 12.05 per cent. of unfreezable water while the seventh internode had 12.66 per cent. This decrease may possibly be due to experimental error, but it may also indicate a reversal of the water relationship in the older stem regions.

The third internode contained more total water per gram of dry weight than any other part of the plant (column 2, table I) and, therefore, less dry weight. There is a smaller percentage of unfreezable water in the third internode than in any of the other internodes. As the stem decreases in total and freezable water, the percentage of unfreezable water increases; or, if the unfreezable water were calculated on a fresh weight basis, it would increase as the dry weight increases. The bud, with the first set of leaves and the first internode, contained more total and freezable water than any of the leaves except the pair at the eighth node. This exception was noted in all plants examined. The eighth pair of leaves appear to be water storage organs. There was a greater percentage, 9.11, of unfreezable water in the bud than in any of the leaves except those at the seventh node which had 9.66 per cent. of unfreezable water. This increased amount of unfreezable water in the leaves at the seventh node is not accounted for, and was seen in all plants examined. The leaves at the fourth and fifth nodes had an average amount of total, freezable, and unfreezable water and were chosen for use in series 2 and 3.

The data in table I are averages of four determinations on three plants where sufficient material was available.

SERIES 2. DETERMINATION OF TOTAL, FREEZABLE, AND UNFREEZABLE WATER IN THE WHOLE LEAF

In this series, the whole leaf with its attached plantlets, was treated as a unit, to determine, if possible, the effect of drying on the amount of total, freezable, and unfreezable water. Leaves from the fourth and fifth nodes were placed in screen wire trays and allowed to dry from 1 to 13 weeks. The drying of the leaf was first observable in the central region along the midrib; it then proceeded toward the edge, until at 10 weeks the central

region of the upper portion of the leaf was tough and brown. The edge and base remained turgid and green (fig. 2). The leaves, with attached plantlets, were chopped, ground, and frozen in the manner already described.

TABLE I

TOTAL, FREEZABLE, AND UNFREEZABLE WATER IN LEAVES AND INTERNODES OF *Bryophyllum*
EXPRESSED IN GRAMS PER GRAM OF DRY WEIGHT, AND THE PERCENTAGE
UNFREEZABLE WATER

MATERIALS	TOTAL WATER IN GM./GM. DRY WT.	FREEZABLE WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN PER CENT.
	gm.	gm.	gm.	%
Bud with first two leaves and internode	8.447	7.678	0.769	9.11
2nd leaves	7.770	6.691	0.808	10.40
2nd internode	10.233	9.657	0.575	5.62
3rd leaves	7.355	6.718	0.637	8.66
3rd internode	10.385	9.706	0.678	6.53
4th leaves	6.470	6.101	0.368	5.69
4th internode	9.446	8.723	0.723	7.65
5th leaves	5.395	5.018	0.376	6.98
5th internode	8.367	7.643	0.723	8.64
6th leaves	4.818	4.614	0.204	4.23
6th internode	7.189	6.431	0.766	10.64
7th leaves	6.409	5.789	0.619	9.66
7th internode	3.751	3.276	0.475	12.66
8th leaves	9.471	8.776	0.694	7.33
Basal internodes	2.692	2.368	0.324	12.05

During the first 2 weeks of drying the leaves became limp and lost 16.54 per cent. of their total water. There was a greater amount of total water, 9.026 gm./gm. dry weight, after 3 weeks of drying than in fresh leaves, 7.719 gm. (column 2, table II). During the third week the roots in the notches of the leaves grew more vigorously than at any other time (fig. 1, B). During the next 8 weeks there was from 18 to 58 per cent. more water in the drying leaf than in the fresh leaf on the dry weight basis. It was during this period that shoots of the regenerating plantlets exhibited the most active growth (fig. 1, C). After 11 weeks the plantlets did not noticeably increase in size.

Since the leaf had no access to water, except that present as vapor in the atmosphere, it is evident that internal changes were causing a reduction in the dry weight. This might be expected, since the energy and materials for the production and growth of the plantlets must be derived from the parent leaf. If there were any increase in internal solids as a result of photosynthetic activity in the leaf or the plantlet, it could not have been measured except as a difference between the material produced and that used by the growing plantlets.

There was a greater percentage of unfreezable water in the leaf and young plants after drying for 13 weeks than in fresh leaves (column 5, table II). There was no gradual increase in the unfreezable water as drying progressed, as might have been expected, but rather a decrease from 8.09 per cent. in the fresh leaf to 3.41 per cent. in the fifth week. The percentage of unfreezable water does not appear to increase or decrease in exact proportion to the total or freezable water, or to the dry weight. It only roughly varies directly with the dry weight, and therefore inversely to the freezable water. Thus any equilibrium that is established between the freezable and unfreezable water must vary from day to day independently of the drying process.

It will be noted that during the eighth week there was less total water than during the seventh and ninth weeks (column 2, table II). The roots had completed their growth during the fifth week and the most vigorous growth of the shoots of the young plants took place during the ninth and tenth weeks. The percentage of unfreezable water in leaves that had dried for 8 weeks was higher than at any time from the second to the thirteenth week (column 5, table II). After 11 weeks the leaves dried out rapidly having less freezable and total water, and in the thirteenth week more unfreezable water in gm./gm. dry weight. This rapid drying is probably the factor that caused the plantlets to stop their growth. They could maintain themselves but could not increase in size on the amount of water they could obtain from the parent leaf.

The data in table II are averages of five determinations on each of three leaves, in all of which, if attached, the plantlets were considered a part of the leaf.

SERIES 3. DETERMINATION OF TOTAL, FREEZABLE, AND UNFREEZABLE WATER IN PARTS OF THE LEAF AND PLANTLETS, DURING DRYING

In series 3 determinations were made on the different parts of the leaf, and on the plantlets produced at the notches of the leaf to determine the amount of total, freezable, and unfreezable water in each portion and the changes in the quantitative relation to the drying process. Since the leaves first dry out in the central region and leave a margin of green turgid tissue at the edge and base, the leaf was so sectioned that the central region was examined separately from the edge and base. The leaves that had not dried more than 6 weeks were cut along a vein leading to the fourth notch from the base of the leaf; then along the margin, one-quarter of an inch from the edge, to the apex. After 6 weeks, the leaf was limp and yellow (fig. 1, C), in the central region with a definite line of demarcation between the dry area and the turgid area at the edge and base. Then the leaf was cut, as shown in figure 2, along the solid line.

TABLE II

TOTAL, FREEZABLE, AND UNFREEZABLE WATER IN GRAMS PER GRAM OF DRY WEIGHT,
AND UNFREEZABLE WATER IN PERCENTAGES

MATERIAL	TOTAL WATER IN GM./GM. DRY WT.	FREEZABLE WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN GM./GM. DRY WT.	UNFREEZABLE WATER IN PER CENT.
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	%
Fresh leaf	7.719	7.083	0.625	8.09
1-week-old leaf	6.586	6.055	0.530	8.27
2 " " " "	6.443	6.019	0.423	6.52
3 " " " "	9.026	8.332	0.698	8.05
4 " " " "	12.047	10.892	0.655	5.78
5 " " " "	12.293	11.890	0.402	3.41
6 " " " "	10.745	10.135	0.610	5.81
7 " " " "	11.400	10.567	0.833	7.32
8 " " " "	9.088	8.372	0.717	7.88
9 " " " "	10.420	9.787	0.632	6.07
10 " " " "	11.811	11.093	0.718	6.09
11 " " " "	11.565	10.737	0.827	7.17
12 " " " "	7.802	7.307	0.505	6.44
13 " " " "	5.768	5.061	0.707	12.26

Roots appeared after the second week, and shoots after the fourth week (fig. 1, A, B). The most vigorous growth of the shoots took place during the ninth and tenth weeks. After 10 weeks of drying the central portion of the parent leaf was tough and brown, but the edge and base became even more turgid and remained green. After 12 weeks the central region was brittle dry, and after 18 weeks the whole leaf was so dry that it would crack if picked up by the petiole, and the plantlets would fall off unless the leaves were handled with great care.

In all determinations for the first 14 weeks more total water was found in the edge and base than in the central portion of the leaf on the dry weight basis. The percentage of unfreezable water was greater in the central portion than in the tissues at the edge and base during these 14 weeks, except the fifth week, at which time the tissues contained only 7.19 per cent. in the central portion and 9.32 per cent. in the edge and base (columns 4, 7, table III). This exception is not accounted for. In this series the total water decreased during the ninth and tenth weeks as it did during the eighth week in series 2. In the tissues at the edge and base of the leaf in the eleventh week there was more total water per gram of dry weight than at any other time. This parallels the determination in series 2, varying slightly in the time intervals. The total water in the central region of the leaf, on dry weight basis, did not increase after the tenth week of drying but continued to decrease until the leaf was brittle dry. The data taken in the eighteenth week were entered in table III as the central region of the leaf since there was no green or turgid tissue in the edge and base.

The percentage of unfreezable water was greater in the central portion of the leaf in each determination, with the exception of the fifth week, than in the tissues at the edge and base (columns 4, 7, table III).

The first determination on the plantlets was made after the leaves had dried for 5 weeks. The total water per gram of dry weight is less in the young plants from the fifth to tenth weeks than in the central region or the tissues along the edge and base, but after 11 weeks the plantlets had more total water than the central portion but less than was found in the tissues along the edge and base of the leaf. At 18 weeks the plantlets contained about thirty-two times as much total water as the leaf, on the dry weight basis.

The percentage of unfreezable water in the young plants is greater from the fifth to the eleventh weeks than in the parts of the parent leaf examined (columns 4, 7, 10, table III). From the twelfth to the eighteenth weeks there was a smaller percentage of unfreezable water in the plantlets than in the leaf. The greatest percentage of unfreezable water in the plantlets was found during the fifth and sixth weeks.

The data in table III are averages of four determinations on three leaves where material was available.

MEASUREMENTS ON CUTICLE THICKNESS AND DISTRIBUTION OF STOMATA ON LEAVES

Since *Bryophyllum* has the ability to retain its water to a remarkable degree, the cuticle and stomata were examined to determine their rôle in this water-retention if possible. The cuticle was examined from prepared slides, and the stomata were counted from impressions made of celloidin peels of the upper and lower surfaces of the leaves.

The cuticle of the upper epidermis was $4\ \mu$ thick and that of the lower epidermis only $2\ \mu$. On the upper epidermis of a mature leaf there were 20 stomata per mm^2 and on the lower 46 per mm^2 . On the leaf of a plantlet there were 20 stomata per mm^2 on the upper and 30 on the lower epidermis. The cuticle is no heavier than on most mesophytes. If the average number on the upper epidermis of a mesophyte is 120 per mm^2 and the average for the lower is 140 then the leaves of *Bryophyllum* have fewer stomata than mesophytes. This cuticle and epidermis of the mature leaf and the plantlet could hardly be thought of as responsible for the water-retaining properties of *Bryophyllum*.

TURRELL (22) reports that the ratio of the internally exposed surface to the externally exposed surface of a leaf of *Bryophyllum* is only 7.8. This is the lowest ratio he reported in all of the plants he examined. This feature of the leaf structure may account in part, at least, for the ability of the leaf to hold its water against drying during an 18-week period. The internally

exposed surface, with the reduced number of stomata, may be in part responsible for the water-retaining capacity of the leaf.

Discussion

The distribution of total water in the whole plant, when the leaves and portions of the stem were examined, indicate that more total water per gram of dry weight was contained in the younger portions of the stem than in the leaves attached to those portions (column 2, table I). There was a higher percentage of unfreezable water in the second and third pairs of leaves than in the second and third internodes of the stem. As the stem became drier in the lower internodes the unfreezable water increased (column 5, table I). The bud, with the first set of leaves and the first internode, contained more total water than any of the leaves except those at the eighth node, which appeared to be water storage organs. The plants used were potted during the late autumn, and the leaves at the eighth node were smaller and thicker, never increasing in size as did the younger leaves.

In these plants in which the whole leaf and stem portions were examined, a large amount of total water was accompanied by a small amount of unfreezable water. The freezable water increased as the total water increased but the unfreezable water decreased, and conversely as the total water decreased the unfreezable water increased. In the leaves this relationship was less striking than in the stem.

When the whole leaf was examined during a period of drying, the total water and freezable water appeared to increase from the third to the eleventh week of drying (columns 2, 3, table II). This apparent increase of water must have been due to an actual decrease in the dry weight, since it is highly improbable that the leaves would take up more water from the atmosphere than they could from a plant growing in well-watered soil.

MOTHES (14) states that there is a greater respiration intensity in wilted leaves than in normal ones. He reported that in *Helianthus* there was a translocation of materials from the older leaves to the younger until the older leaves were exhausted. Any translocation that takes place in the detached leaves of *Bryophyllum* must be from one part of the leaf to another or to the young plantlets on the margin of the leaf. Translocation cannot take place to anything outside of the leaf or the plantlets, and the loss in dry weight must be due to respiration. The greatest loss in dry weight, as measured by the increase of total water in grams per gram dry weight, took place during or immediately following the most vigorous growth of the plantlets at the margin of the leaf. During the period of loss in dry weight the freezable water increased but the unfreezable water decreased.

As the leaf dries, in the manner described in series 2, the unfreezable water decreases until about the fifth week of drying (column 5, table II).

This decrease is not a steady decrease but an irregular one. After the fifth week the percentage of unfreezable water increases, not steadily but irregularly, until at 13 weeks it reaches 12.26 per cent., which is the highest found in the drying leaves. This final percentage is 4.17 per cent. above that of the fresh leaves examined 13 weeks before. The total water per gram of dry weight is 5.768 gm. after 13 weeks of drying as compared with 7.719 gm. in the fresh leaf (column 2, table II). This is one-fourth less total water in the 13-weeks-old leaf than in the fresh leaf on the dry weight basis.

Thus, it might at first appear that there was an increase in the percentage of unfreezable water in the process of severe drying. Almost one-half of the increase took place in the last week. The percentage of unfreezable water was 6.44 per cent. after twelve weeks of drying or 1.65 per cent. less than in the fresh leaf. It was between the determinations made the twelfth and thirteenth weeks that the most rapid drying took place, and the percentage of unfreezable water increased most during the thirteenth week. It therefore appears that the increase in percentage of unfreezable water is not responsible for the capacity of *Bryophyllum* leaves to retain water during severe drying, but rather increased merely as the result of this drying process.

The results of the measurements in series 3 indicate a shift of the total water from the central portion of the leaf to the edge and basal tissues. In the fresh leaf there were 5.611 gm. of total water in the central portion and 6.160 gm. in the edge and base per gram of dry weight (columns 2, 4, table III). At 6 weeks there were 7.405 gm. in the central portion and 10.257 gm. in the edge and base, and at 11 weeks 6.56 gm. in the central region and 13.269 gm. of total water in the edge and base per gram of dry weight. The ratios of total water in the marginal and basal tissues to the central tissues are: in fresh leaves, 1.09; after 6 weeks, 1.37; 11 weeks, 2.02; and at 14 weeks, 8.65. The ratios of grams of unfreezable water in the edge and base to the central region are: in fresh leaves, 0.72; after 6 weeks, 0.43; 11 weeks, 0.74; and 14 weeks, 0.27. Thus the ratio of unfreezable water in the edge and base of the leaf to the central portion decreases markedly as the leaf dries. Since the edge and base resist drying longer than the central region and if the unfreezable water was responsible for this resistance, then the ratios should increase. This was found not to be the case.

The movement of the water from the central region to the edge and basal tissues will maintain the young plants at the margin of the leaves. The increased unfreezable water in the central tissues of the leaf seems to be the result of the shift of the total water to the marginal and basal tissues rather than a means for the shift. The shift may be caused by greater osmotic values of the tissues at the margin. FREELAND (4) found in some of the leaves of *Bryophyllum* that were producing new plants at the margin, a greater osmotic value at the margin than in the central region.

In the plantlets the total water in grams per gram of dry weight was observed to be less than that of the leaf from the fifth to the eleventh week (column 8, table III). However, at the end of 18 weeks there were 4.213 gm. of total water in the plantlets and only 0.130 gm. in the parent leaf on the dry weight basis. The plantlets remained turgid and green while the parent leaf was brittle dry. The percentage of unfreezable water in the plantlets decreased from 11.81 per cent. in the very young plants to 5.73 per cent. after drying for 7 weeks, then increased to 10.14 per cent. after drying for 13 weeks (column 10, table III). If the unfreezable water were responsible for the ability of these plantlets to retain their water it should increase, if not from the first determination then certainly after 13 weeks of drying. This was not the case. In the plantlets there is less relation between the severe drying and the unfreezable water than in the drying leaf.

It appears that the unfreezable water in *Bryophyllum* leaves increases with the severe drying of the leaves and cannot be responsible for the water-retaining capacity of the leaf and plantlets. The unfreezable water shows less correlation to the drying in the plantlets than in the parent leaf.

Summary

1. The term freezable water is used to designate that state of water referred to by some authors as "free" water, and unfreezable water as that referred to as "bound" water.

2. Since measurements of unfreezable water in the tissues of plants had been frequently employed in attempts to interpret the changes occurring in cold hardening, and very little had been done on drought resistance in relation to the unfreezable water content of tissues it seemed desirable to determine the total, freezable, and unfreezable water in *Bryophyllum* leaves during severe drying.

3. In the whole plant in general there was a correlation between the age of the tissue examined and the percentage of unfreezable water it contained, that is, the older the tissues the greater the percentage of unfreezable water. The bud, however, contained less unfreezable water than did tissues of intermediate age.

4. As the leaf dries out the percentage of unfreezable water increases as a result of the loss of freezable water by evaporation. The increase in the unfreezable water does not seem to the writer to be responsible for the water-retaining ability of the leaf but rather a result of the drying.

5. In the plantlets, where there is the greatest ability to retain the water against atmospheric drying, there is less correlation between the unfreezable water and the drying than in the drying of the parent leaf. The youngest plants on the margin of the leaves have the greatest percentage of unfreezable water.

6. The reduced internally exposed surface and the reduced number of stomata may be responsible for the ability of *Bryophyllum* leaves to retain their water.

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